The use of faecal cuticle examination to determine the dietary composition of koalas

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ABSTRACT

Captive koalas were provided with specified proportions of known browse species. Faecal pellets collected from each koala were analysed for cuticle fragments to determine the accuracy of this method for estimating the species composition of the diet of koalas. Food species were consistently observed in faecal pellets collected 34 h after presentation of browse and remained represented in pellets collected up to 154 h post-feeding. Faecal pellet examination provided an accurate means of describing the species composition of the diet of the koalas for the species of browse offered.

Key words: Koala diet, Faecal cuticle examination.

INTRODUCTION

The digestive physiology of koalas (Cork 1990), their preferred browse species (Hindell *et al.* 1985; Martin 1985) and the factors which influence their leaf selection (Ullrey *et al.* 1981; Hume and Esson 1993) have been documented. The studies of Hindell and co-workers (1985) and Hindell and Lee (1987, 1988) suggested that the comparison between percentage use of species and the relative abundance of those species at a site could be used to determine tree preference indices; these reports concurred with earlier work by Eberhard (1972, 1978) and Martin (1985) that day time tree occupancy reliably predicted preference for food trees in free-ranging koalas. However, Tun (1993) and Hasegawa (1995) indicated that, based on the analysis of diet composition from faecal cuticle fragment identification, this was not the case for the populations of koalas they examined in Queensland.

There has been widespread use of the microscopic analysis of faecal material to determine dietary composition of herbivores and folivores (Storr 1961; Stewart 1966; Fitzgerald 1984; MacLennan 1987; Pahl 1987). The stomatal complex and arrangement of subsidiary and guard cells on the cuticle of certain *Eucalyptus* leaves was shown by Carr *et al.* (1971) and Carr and Carr (1985) to be distinctive enough to separate species according to these features. The first detailed description of the use of this approach to ascertain the diet of koalas appears to be that of Tun (1993); this method has since been used to determine the diet of koalas in other areas of south-east Queensland (Hasegawa 1995) and northern New South Wales (Lloyd and Fisher 1997).

The use of microscopic analysis of faecal material for diet determination assumes that all plant cuticle fragments remaining in koala faeces have the same probability of being detected. Further, it assumes that the proportions of all plant material consumed will be accurately represented in the faecal pellets. The work reported here evaluated the accuracy of the technique for describing proportions of food species eaten by captive koalas and determined the time period between the consumption of species and their appearance in faecal material, as well as the retention of these species post-consumption.

METHODS

Feeding regime

Captive koalas (two male, two female) housed at The University of Queensland were maintained *ad-libitum* on selected fresh browse, comprising *Eucalyptus tereticornis, E. racemosa, E. melanophloia, E. seeana, E. resinifera, E. major, E. crebra, E. microcorys, E. robusta* and *Corymbia citriodora.* The koalas were housed in galvanized steel and wire mesh enclosures mounted on cement. The enclosures measured 4 m × 2.5 m × 2 m tall and were fully covered. Each koala was supplied with a pre-determined proportion of the browse species which was altered each week of the study. During the first week, *E. tereticornis* was excluded from the species provided to the koalas. Throughout the second week the only species provided was *E. tereticornis.* In week 3, one male and one female were fed *E. propinqua* exclusively; the other two koalas received only *E. microcorys.* In the fourth week, all four koalas received equal proportions of *E. tereticornis, E. resinifera* and *C. citriodora.*

Eucalyptus branches presented to koalas as food, draw water from their containers and can increase in weight; alternatively, some braches which are fully hydrated when presented to the koalas may lose water through evaporation. As a result, the weight of branches before and after presentation does not reflect the amount of leaf material consumed by the koalas. Each branch to be presented to each koala was, therefore, individually labelled and photographed prior to being placed in the feeding containers within the koalas' enclosures at 0800 hr each day. Branches removed were similarly photographed and each koala was weighed between the removal of the previous day's food supply and the presentation of the new browse each day. In this manner the proportions of the food species eaten each day were able to be estimated.

Pellet collection and diet analysis

All faecal pellets produced by each koala were collected each hour between 0600 hr and 1000 hr and also between 1600 hr and 2200 hr. In the intervening periods, pellets were collected each 2 to 6 hours. Pellets collected were counted and placed in resealable plastic bags, weighed and stored at –20°C.

Reference slides

Species reference slides were prepared according to the method of Tun (1993). Briefly, leaf segments (1 cm × 1 cm) from all the feed species used in the study were fixed (70% ethanol: 5% glacial acetic aid: 5% formalin at 90:5:5 v:v) then boiled in hydrogen peroxide: glacial acetic acid (6:1) in a fume hood to remove the mesophyll and separate the cuticle layers. After washing with water, the adaxial and abaxial cuticle layers of the specimens were removed with forceps and stored in 60% ethanol. These layers were then stained in aqueous gentian violet for 30 sec, dehydrated by washing in alcohol, cleared in xylene and mounted under cover slips in xam (Merck, Kilsyth, Victoria) on microscope slides. These slides were examined under a light microscope at ×10 and ×40 using an eye-piece micrometer as reference for each species' identification.

Tun (1993) provided a complete description of the diagnostic features used to distinguish *Eucalyptus* and other potential koala fodder species on the basis of microscopic analysis of leaf material. Briefly, adaxial and abaxial surfaces were examined since some species are dorsiventral with respect to stomata on these surfaces, having no stomata on the adaxial surface and only a simple cuticular pattern. Two basic types of stomatal complex were evident and although Metcalfe (1979) suggested epidermal cell shape to be of limited diagnostic value, Tun (1993) found mean cell size to be variable between species and useful for this purpose. The mean size of the stomatal complex, together with density, was confirmed by Tun (1993) to be a highly reliable diagnostic character and the number of subsidiary cells was also useful for separating some species. The density and mean length of the stomatal complex are perhaps the best diagnostic features for this type of analysis.

Preparation of slides from faecal pellets

Preparation of faecal pellet material for examination also followed the method of Tun (1993). Pellets chosen for analysis were placed in centrifuge tubes (10 ml), washed with tap water and several drops of detergent (Decon) and left overnight on an orbital shaker (Bio-Line, Edwards Instrument Co. New South Wales) at 37°C. These tubes were then shaken and the pellets crushed using forceps prior to the tubes being centrifuged (3 000 rpm for 3 min). The supernatants were poured off and the tubes filled with water, shaken and centrifuged again. This washing was repeated three times after which the tubes were filled with 4% sodium hypochlorite and left until any remaining mesophyll had been removed (up to five days). The bleached material was then centrifuged and washed as before and alcohol (50 %) added to the tubes prior to staining.

A small amount (0.5 ml) of the bleached material was adhered to a microscope slide using Mayer's albumen and stained with gentian violet. This preparation was then dehydrated, cleared in xylene and mounted under a coverslip using xam.

These permanent slides were compared with the reference slides at ×40 magnification by systematic traversing to avoid double counting of fragments. In each slide, a sample of 100 fragments was identified and the frequency of occurrence of each species recorded. All leaf fragments were able to be identified using the method of Tun (1993). Stem and young leaf fragments which were unable to be identified were not included in the species composition data. One pellet from each collection period (described above) from each koala was examined in this manner in accordance with the method of Tun (1993), giving a close approximation of the time at which those species which had been consumed subsequently appeared in the faeces.

Accuracy of estimation of pellet composition

Once slides from all koalas for all days were prepared and examined, the accuracy of the slide scanning procedure was tested. Pellets which were comprised of 100% of one species were combined together with similar pellets (equal mass and from the same koala) for one other species in set proportions and a composite slide prepared to determine whether the proportions of material found in other slides accurately reflected the composition of the pellets from which the slides were made. The slides which were prepared in this manner were examined by an observer who was unaware of the pellet proportions (and hence species proportions) to determine the accuracy of the scanning.

RESULTS

Pellet collection

Average pellet production per koala, as shown in Table 1, was calculated to be 175 ± 41.9 pellets per day. Few pellets were produced between 1000 hr and 1600 hr. The disturbance of feeding procedures coincided with a slight but insignificant increase in the number of pellets collected for this period of daylight. For analysis purposes, collection of pellets was separated into daylight (0600–1900 hr), night (1900–0600 hr) and the period of feeding and weighing (disturbance) (0700–0900 hr), data for which were included in "daylight" (Table 2).

Table 1. Koala mass and pellet production for captive koalas in the present study.

Koala	Mass kg ± sd	Pellet production number per day ± sd	Pellet mass per pellet ± sd
Male 1	5.87 ± 0.04	142 ± 24	0.92 ± 0.21
Female 1	5.80 ± 0.32	186 ± 42	0.44 ± 0.03
Male 2	6.95 ± 0.07	205 ± 38	0.45 ± 0.09
Female 2	2.65 ± 0.07	167 ± 35	0.39 ± 0.07

Table 2. Pellet production rates (pellets per hour) for captive koalas during the periods of Daylight (0600–1900 hr), Night (1900–0600 hr) and Disturbance (0700–0900 hr) in the present study.

Koala	Daylight	Night	Disturbance
Male 1	4.8 ± 3.1	7.4 ± 1.9	5.4 ± 4.7
Female 1	6.4 ± 2.6	7.9 ± 2.8	6.5 ± 4.8
Male 2	1.8 ± 1.5	12.4 ± 2.7	10.7 ± 2.8
Female 2	4.9 ± 1.8	8.4 ± 1.9	8.0 ± 5.6

Identification of species and passage of dietary components

There was close agreement between the ratio of species fed to the koalas and the relative frequency of occurrence of those species in the slides produced from faecal pellets from those koalas. Species consumed generally appeared in faecal material after 34 h and remained present in pellets for up to 154 h ($\bar{X}$ = 111.4 h sd = 41.2 h). In two cases, a small proportion (2%, day 15, Fig. 1 and 8%, day 1 Fig. 2) of *E. tereticornis* was identified in a faecal sample 10 h (Fig. 1) and 11 h

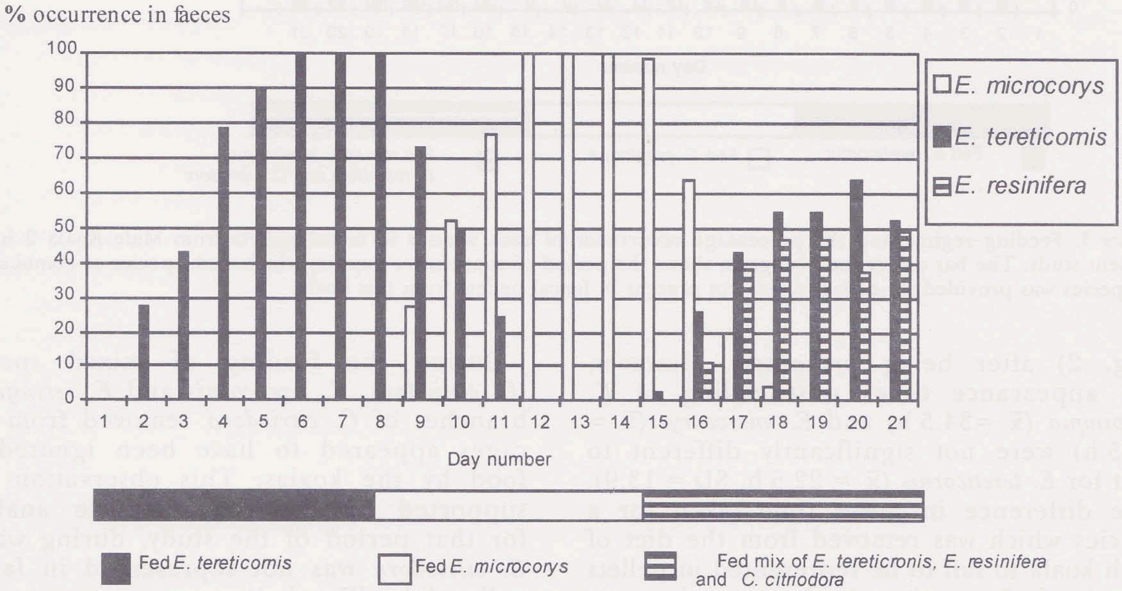

Figure 1. Feeding regime and the percentage occurrence of each *Eucalyptus* species in faecal pellets from Male Koala 1 in the present study. The bar under the histogram shows the period of consecutive days on which each species or combination of species was provided. *C. citriodora* was not present in faecal pellets from this koala.

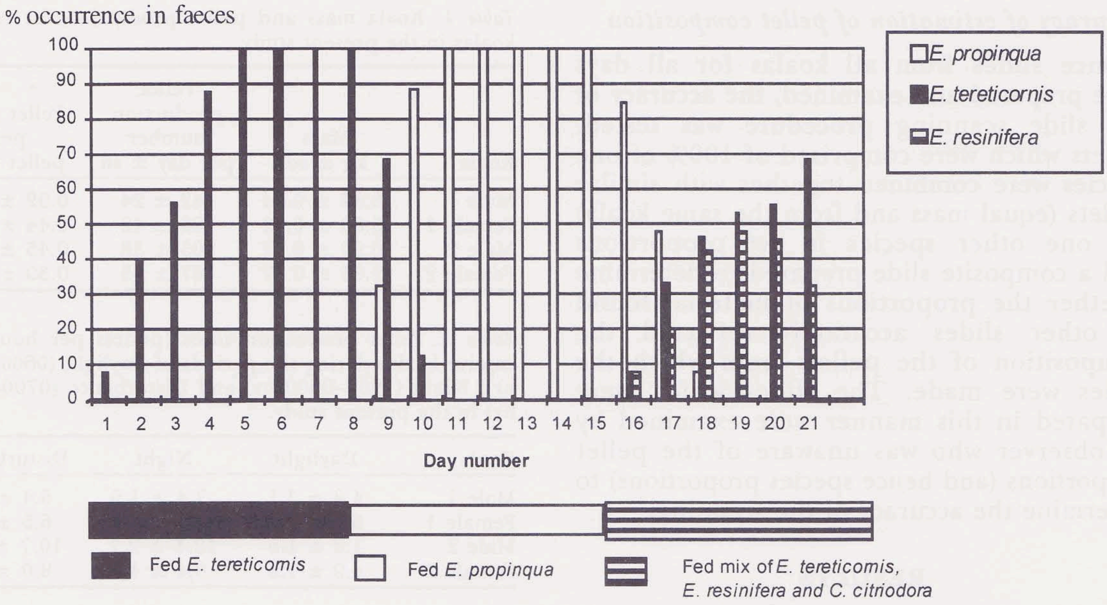

Figure 2. Feeding regime and the percentage occurrence of each species in faecal pellets from Female Koala 1 in the present study. The bar under the histogram shows the period of consecutive days on which each species or combination of species was provided. *C. citriodora* was not present in faecal pellets from this koala.

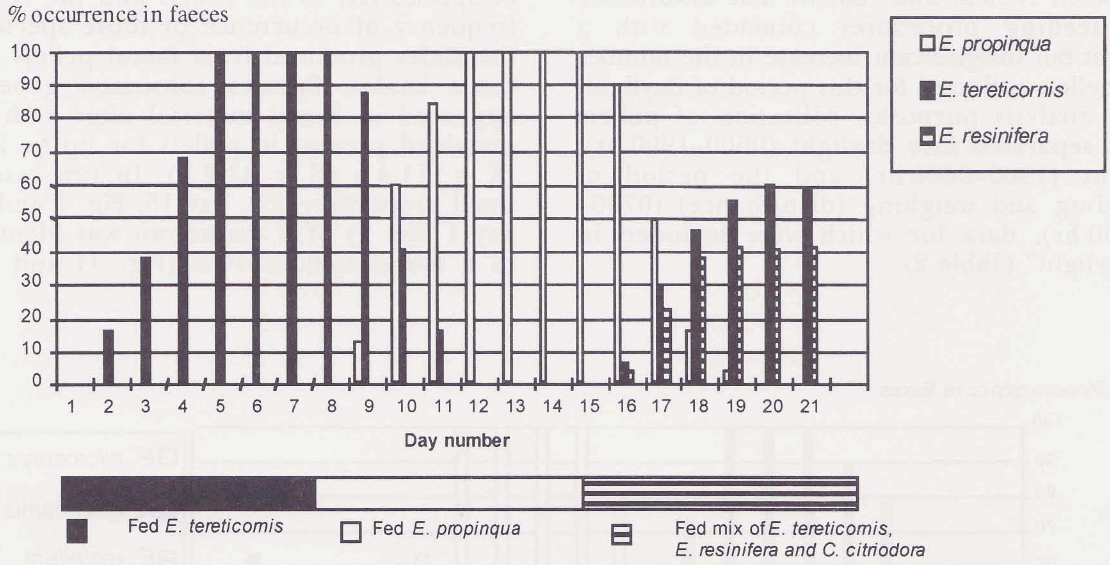

Figure 3. Feeding regime and the percentage occurrence of each species in faecal pellets from Male Koala 2 in the present study. The bar under the histogram shows the period of consecutive days on which each species or combination of species was provided. *C. citriodora* was not present in faecal pellets from this koala.

(Fig. 2) after being presented. However, the appearance times post-feeding of *E. propinqua* ($\bar{x}$ = 34.5 h) and *E. microcorys* ($\bar{x}$ = 34.5 h) were not significantly different to that for *E. tereticornis* ($\bar{x}$ = 22.5 h, SD = 13.9). The difference in mean time taken for a species which was removed from the diet of each koala to fail to be represented in pellets examined from that koala was also not significant (*E. tereticornis* $\bar{x}$ = 119 h, sd = 10.7, *E. propinqua* $\bar{x}$ = 137 h, sd = 17, *E. microcorys* $\bar{x}$ = 137 h, sd = 17, Figs 1–4).

During the feeding of mixed species (*C. citriodora, E. tereticornis* and *E. resinifera*), branches of *C. citriodora* removed from the cages appeared to have been ignored as food by the koalas. This observation was supported by the faecal cuticle analysis for that period of the study, during which *C. citriodora* was not represented in faecal pellet slides (Figs 1–4).

Male koala 1 produced pellets which were significantly larger and of greater dry mass

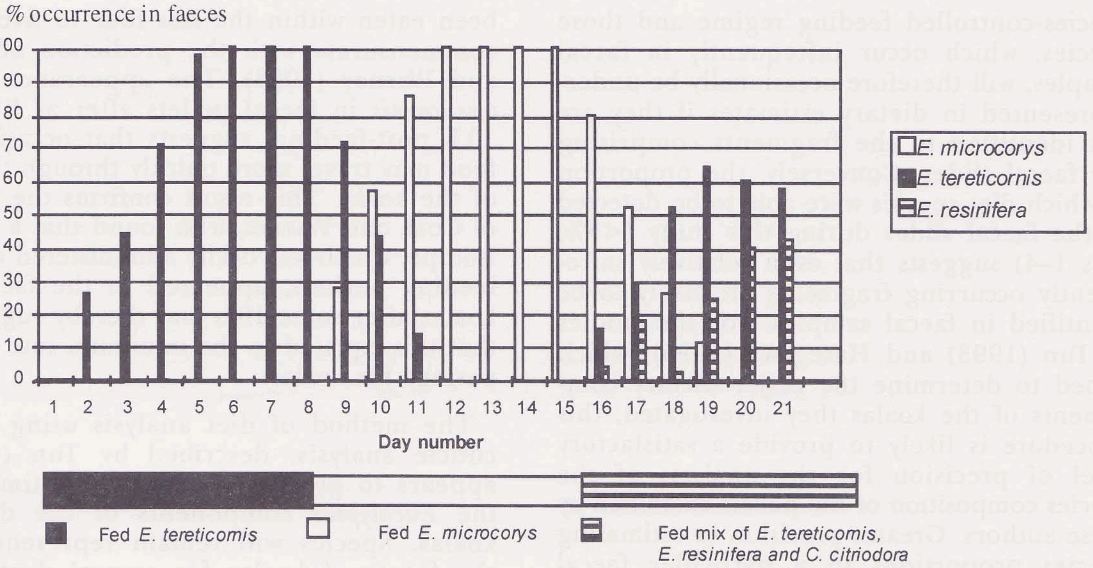

Figure 4. Feeding regime and the percentage occurrence of each species in faecal pellets from Female Koala 2 in the present study. The bar under the histogram shows the period of consecutive days on which each species or combination of species was provided. *C. citriodora* was not present in faecal pellets from this koala.

($t_{24,\ 24} = 180$, $P < 0.05$, Table 1) than the other koalas in the study. Total daily faecal dry matter ranged between 21.55 g $kg^{0.75}$ day^{-1} (Male koala 2) and 34.65 g $kg^{0.75}$ day^{-1} (Male koala 1), suggesting some variation to exist in either the amount of food consumed, or the digestibility of dry matter consumed, between the koalas in the present study.

Accuracy of species proportions identification in slides prepared from faecal pellets

Pellets collected at day 7 and day 14, which were each comprised of 100% of one species (*E. tereticornis* or *E. microcorys*) were combined in pellet proportions as follows: sample a: 1 pellet of *E. tereticornis* and 1 pellet of *E. microcorys*, sample b: 1 pellet of *E. tereticornis* and 2 pellets of *E. microcorys*, sample c: 2 pellets of *E. tereticornis* and 1 pellet of *E. microcorys*. All pellets in each sample were combined and analysed as a single sample, according to the method described previously, without the proportions being revealed to the analyst prior to the test. The resulting proportions of cuticle fragments identified in the resulting slides (sample a: 47% *E. tereticornis,* 53% *E. microcorys*; sample b: 34% *E. tereticornis,* 66% *E. microcorys;* sample c: 66% *E. tereticornis,* 34% *E. microcorys*) were accurate estimations (to within 3%) of the samples from which they were drawn.

DISCUSSION

Significant associations between the use of species of trees by koalas for roosting and the defoliation of these species have been observed (Martin 1985), and there is apparent agreement between biologists and the keepers of captive koalas as to the browse preferences of koalas in general (Hume and Esson 1993). Although there has been agreement that diet preferences of koalas are reliably reflected in roost preferences (Hindell *et al.* 1985; Melzer 1994; Ellis *et al.* 1995), reports which have analysed the diet of koalas using cuticle analysis do not support this assumption; Tun (1993) and Hasegawa (1995), instead concluded that roost tree selections made by koalas do not resemble their dietary selection.

The controlled feeding used in this examination ensured that mis-identification of fragments of cuticle in the faeces of koalas was unlikely. Only those species presented to the koalas were identified in the faecal pellets produced, and the species selected were reliably distinguished using the key developed by Tun (1993).

The result of the experiment to test for the accuracy of food species-proportion estimates indicates that the method provides a relatively accurate description of the species composition of faecal pellets. The species used in this study were readily discernible, yet an error of precision in the order of ± 3% was evident in one sample comprised of two species (Sample a). This error may have resulted from insufficient mixing of the material comprising the slide, or may reflect the level of precision of the technique, at least for the species used. Many more species are often present in faecal pellets collected from koalas which have not been subjected to a species-controlled feeding regime and those species, which occur infrequently in faecal samples, will therefore occasionally be underrepresented in dietary estimates if they are not identified in the fragments comprising the faecal slides. Conversely, the proportion at which diet species were able to be detected in the faecal slides during this study (<5%, Figs 1–4) suggests that even relatively infrequently occurring fragments are likely to be identified in faecal samples. For the studies of Tun (1993) and Hasegawa (1995), which aimed to determine the major dietary components of the koalas they investigated, this procedure is likely to provide a satisfactory level of precision for the analysis of the species composition of the pellets examined by these authors. Greater precision in estimating species proportions in a particular faecal pellet sample may be obtained by counting a larger sample of fragments from each slide, thereby increasing the likelihood that infrequently-occurring fragments will be identified. However, a more representative estimate of the diet of koalas is likely to be obtained by analysing a larger sample of faecal pellets collected over a period of time, so in practice the identification of 100 fragments per slide provides an adequate estimate of major components in a particular sample and provides the opportunity to evaluate more samples for a particular level of input.

Buetel (1990) observed a tendency for koalas to defecate once aroused. However, the relationship between activity and patterns of pellet deposition for free-ranging koalas is reported to be weak (Ellis *et al.* 1998) and no consistent pattern of deposition related to disturbance was evident in the current study. The sample of four koalas, over 24 days of collection included one individual (Male Koala 2) for which there appeared to be increased pellet deposition during the period of disturbance, hence it is possible that captive handling procedures may influence the collection of data in some cases. This result is similar to that reported by Cork and Warner (1983) who commented that few 3 h collection periods passed without significant numbers of pellets being produced by the koalas in their study.

The appearance of food species in faecal pellets produced 34–35 h after food being offered indicates that fresh pellets will provide information on the species consumed by a koala no earlier than 34 h prior to pellet collection. The pellet may contain remnants of food consumed six days previously, however, it is likely that most material will have been eaten within the last four to five days, commensurate with the prediction of Cork and Warner (1983). The appearance of *E. tereticornis* in faecal pellets after as little as 10 h post-feeding, suggests that occasionally food may travel more quickly through the gut of the koala. This result confirms the report of Cork and Warner, who found that a radioisotope, which was orally administered during feeding periods, appeared in the faeces of koalas after some 10 h and thereby suggested that this equated to the minimum rate of gut passage for koalas.

The method of diet analysis using faecal cuticle analysis, described by Tun (1993), appears to provide an accurate estimate of the *Eucalyptus* components of the diet of koalas. Species will remain represented in the faeces of koalas for several days after the koalas have eaten them (Figs 1–4), hence an accurate description of the proportion of species eaten by free-ranging koalas will require the collection of a sample containing pellets produced over several days. Since the proportions of each species present in pellets will depend not only on the proportions eaten, but also on the period which has elapsed since they were consumed, a description of the proportion of species eaten will require a larger sample than was described by Tun (1993).

The absence of *C. citriodora* in faeces of koalas in this study indicates that either this species is not an appropriate fodder species for captive koalas, that the koalas used in the study did not consider it as suitable food, or that in the presence of the other species available during the period of the study it was not favoured. This is despite numerous sightings of koalas in this tree species in the Brisbane area. Analysis of pellets from free-ranging koalas which are observed to utilize this species may provide more information on its role in the ecology of the koalas which utilize it.

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